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PATENT

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Sir:

Enclosed is a certified copy of Priority Document 98201392.2 filed April 29, 1998, for the above-referenced case.

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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

98201392.2

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

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Page 2 de l'attestation

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Novel CD40 interacting proteins

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NOVEL CD40 INTERACTING PROTEINS

The inv ntion relates to novel CD40 binding proteins, which can be used as modulators of the CD40 signalling pathway and/or the CD40-induced Nuclear factor kappa B (NF-κB) activating pathway and are thus useful in th treatment of CD40 related diseases (e.g. inflammatory diseases) and/or NF-κB related diseases and/or in the improvement of anti-tumour treatments.

The invention also relates to nucleic acids coding for said novel CD40 interacting proteins.

The invention relates further to the use of polypeptides derived from these CD40 interacting proteins in the treatment of CD40 and/or NF-κB related diseases and/or cancer.

Furthermore, the invention concerns pharmaceutical preparations comprising the novel CD40 interacting proteins or polypeptides derived from these proteins.

CD40 is a receptor of the TNF- receptor superfamily (Banchereau *et al.*, 1994), which is expressed at the surface of B-cells, antigen presenting cells (APC), and several non-haematopoetic cells such as endothelial cells (Hollenbaugh *et al.*, 1995), epithelial cells (Galy & Spits, 1992), fibroblasts (Fries *et al.*, 1995) and keratinocytes (Gaspari *et al.*, 1996). The ligand for CD40, CD40L, occurs mainly on activated T-cells. Up to now, the role of CD40 was mainly studied in the context of the T-cell APC / B-cell interaction (for a review, see Noelle, 1996). Amongst others, the CD40-CD40L interaction seems to be important for the T-cell mediated immunity and for primary and secondary humoral immune respons. These findings were confirmed by xperiments in mouse models, wh re one was abl to show that

treatm nt with anti-CD40L antibodies resulted in blocking of the development of mouse equivalents of human autoimmun diseases such as arthritis (Durie *et al.* 1993) , oophoritis (Griggs *et al.*, 1996) and multiple sclerosis (Gerritse *et al.*, 1996).

Activation and transduction through the CD40 pathway is in a large part responsible for B cell activation and accordingly, the humoral immune response.

Apart from NF-κB, factors that can be activated by stimulation of CD40 are NF-AT (Francis *et al.*, 1995) c-Jun, ATF-2 and IRF-1 (Karmann *et al.*, 1996). All these factors play an important role in inflammation.

The CD40L induced signal transduction is, as for the case of TNF, mediated by the binding of TNF-Receptor Associated Factors (TRAF's) to the cytoplasmic domain of the receptor. Chaudhuri *et al.* (1997) demonstrated that, at least in human B cell lines, CD40 and TRAF2 are constitutively associated with each other, and that this association is inhibited by CD40 mediated signals. Apart from the binding with TRAF 2, the cytoplasmic domain of CD40, which consists of 62 amino acids at positions 196-257 (mature human CD40 - numbering according to Kashiwada *et al.*, 1998), is known to associate with TRAF3, TRAF5, TRAF6 and Janus kinase 3. TRAF 6 binds to the amino-terminal cytoplasmic tail of CD40 at positions 210-225, although the possibility can not be excluded that full association of TRAF6 with CD40 may also require the carboxy-terminal part at positions 226-249 (Ishida *et al.*, 1996) . TRAF 2, TRAF3 and TRAF5 bind to the carboxy-terminal CD40 cytoplasmic domain at positions 226-249 (Ishida *et al.*, 1996).

Stimulation of CD40 results in activation of protein kinases, NF-κB, the mitogen-activated protein kinase and Janus kinase 3 / signal transducer and activator of Transcription 3. Moreover, stimulation of CD40 mediates critical biological effects in B cell growth, survival and differentiation.

It is known that TRAF2 and TRAF5 play a role in NF-κB activation in signalling through CD40, as well as TNF-RI, TNF-RII, CD30 and lymphotoxin β receptor. TRAF6 participates in NF-κB activation signalled by CD40 and IL-1 receptor.

In addition to these data in WO 96/16665 and WO 96/28568 are disclosed a TRAF like protein that binds to the cytoplasmic domain of CD40.

Surprisingly, it is shown in this invention that two other proteins exist interacting with the cytoplasmic domain of CD40. Even more surprisingly, none of these proteins shows significant homology with one of the known CD40 interacting proteins, neither is there homology between the two proteins themselves. These proteins should therefore be considered as two new classes of CD40 interacting proteins

One aspect of this invention is to offer said novel proteins to modulate and/or inhibit CD40 signalling and/or CD40-induced NF-κB activation.

One embodiment of the invention is a protein with SEQ ID NO.2. Another embodiment of the invention is a protein with SEQ ID NO.4. A further embodiment of the invention concerns a protein with SEQ ID NO.6.

A further aspect of the current invention is the use of above mentioned proteins, or biologically active fragments of these proteins, to modulate and/or inhibit CD40 signalling and/or CD40-induced NF-κB activation.

Another aspect of the invention is the use of above mentioned proteins or biologically active fragments of these proteins to screen for compounds that interfere in the interactions of said proteins or fragments with other compounds of the CD40 related signalling pathway.

Another aspect of the invention consists of DNA molecules encoding for the above mentioned proteins.

The invention also relates to a pharmaceutical composition comprising one or more of the above mentioned proteins or fragments in a biologically active amount for the treatment of CD40 and/or NF-κB related diseases such as atherosclerosis, arthiritis, multiple sclerosis, systemic lupus erythematosus, graft rejection and the like.

In another aspect the present invention relates to a pharmaceutical composition comprising one or more compounds obtainable by the above mentioned screening method for the treatment of CD40 and/or NF-κB related diseases such as atherosclerosis, arthiritis, multiple sclerosis, systemic lupus erythematosus, graft rejection and the like.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: schematical representation of CRAP (=CD40 receptor associated protein) and the deletion mutants of CRAP used in two hybrid screening assays. The deletion mutants consist of the following amino acids of the original CRAP sequence: 54 to 362 (4F2), 54 to 273 (4F2d3), 54 to 236 (4F2d2) and 54 to 140 (4F2d1).

Figure 2: Northern blot analysis of (a) human tissue, using a human CRAP probe; (b) adult mouse tissue, using a mouse CRAP probe; (c) embryonic mouse tissue, using a mouse CRAP probe. Th_ hybridization of GAPDH is used as a control.

DEFINITIONS

The following definitions are provided in order to illustrate and define the meaning and scope of the various terms used in the current description.

The term "treatment" or "treating" or "treat" means any treatment of a disease in a mammal, including :(1) preventing the disease, that is, causing the clinical symptoms of the disease not to develop; (2) inhibiting the disease, that is, arresting the development of the clinical symptoms; and/or (3) relieving the disease, that is, causing the regression of clinical symptoms.

The term "effective amount" means a dosage sufficient to provide treatment for the disease state being treated. This will vary depending on the patient, the disease and the treatment being effected.

"Capable to interact" means that a protein can form a complex with another protein, as can be measured using a yeast two-hybrid system, or with co-immunoprecipitation, or with equivalent systems known to people skilled in the art.

"Functional" protein or fragment means a protein or fragment that is capable to interact with the cytoplasmic part of CD40, or with another protein of the CD40 and/or NF-κB related pathway.

"Homology to TRAF-proteins" means that the typical structural features found in the current TRAF prot ins (TRAF1 - TRAF6) are present. These features compris a RING finger motif at the amino terminus followed by five or more zinc fingers and a so-called TRAF domain known to a person skilled in the art.

The "cytoplasmic part of CD40" means a part comprising the 62 carboxy terminal amino acids of human CD40 (amino acid 216-277; Stamenkovic *et al.* 1989), or the homologous mouse sequence, or another homologous sequence with a similar biological activity.

"Nucleic acid" means genomic DNA, cDNA, double stranded or single stranded DNA, messenger RNA or any form of nucleic acid known to the people skilled in the art.

"Compound" means any chemical or biological compound, including simple or complex inorganic or organic molecules, peptides, peptido-mimetics, proteins, antibodies, carbohydrates or nucleic acids, that interferes with the interaction of a protein depicted in SEQ ID NO. 2, 4 or 6 with a compound of the CD40 and/or NF-κB related pathway.

EXAMPLES

Example 1: isolation of the CD40 interacting proteins

Yeast two-hybrid screening.

The two-hybrid screening was performed by the interaction trap cloning m thod, which is often referred to as the LexA two-hybrid system (Gyuris *t*

al., 1993). The DNA encoding the cytoplasmic part of CD40 (62 amino acids, from residu 216 to 277, where th open reading frame ends, according to the sequence and numbering as given in Stamenkovic *et al.* (1989)) was generated by PCR and inserted into the EcoRI-SalI digested pEG202 vector (Gyuris *et al.*, 1993), in frame with the LeXA DNA-binding domain (hereinafter the "bait plasmid"). Screening was performed using a HeLa cell fusion library in the plasmid pJG45 (hereinafter the "prey plasmid"), that was obtained from the laboratory of R. Brent (Harvard Med. School, Boston, MA, USA). Transformation of EGY48 yeast (MAT alpha, *his3, trp1, ura3-52, leu2::pLeu2-LexAop6*) with the prey plasmid, the bait plasmid and the p8op-LacZ (Clontech) reporter plasmid was performed by the Lithium Acetate transformation method (Gietz *et al.*, 1995). The two-hybrid screening was conducted as described in the manual distributed by the laboratory of R. Brent (published in "Gene probes- A practical approach, Oxford University press").

Results of the two-hybrid screening.

Yeast containing bait plasmid and lacZ reporter plasmid was transformed with 20 microgram prey library plasmid and plated on glucose medium lacking tryptophan, histidine and uracil, to select for the presence of all three plasmids. In total, approximately $1.5x10^6$ transformants were obtained. The transformants were harvested and frozen at -70°C in a glycerol solution (65% v/v glycerol; 0.1 M $MgSO_4$, 25 mM Tris pH 7.4). From these stocks, $20x10^6$ colony forming units were plated on galactose medium lacking leucine,

tryptophan, histidine and uracil, to screen for protein-protein interaction. Yeast colonies growing on the latter s lective m dium were further check d for interaction by screening for blue/white staining on medium containing X-gal and galactose. The colonies displaying the following phenotype were picked for further analysis: i) no growth on glucose containing medium lacking leucine, ii) growth on galactose containing medium lacking leucine, iii) white on medium containing glucose and X-gal, iv) blue on medium containing galactose and X-gal.

Plasmids were isolated from the yeast with the proper phenotype. Analysis of the obtained prey plasmids revealed that the entire screening had finally resulted in the isolation of three different cDNA inserts. Sequencing of the clones showed that, in addition to a partial cDNA for TRAF3, we had isolated two novel cDNA's, termed CRAP and 4C4.

Isolation of the full length cDNA

Full length human CRAP cDNA was obtained by screening a HUVEC cDNA library, made in the laboratory, with the CRAP fragment as probe. A cDNA of about 2 kb was isolated, with an open reading frame of 1086 nucleotides long, encoding for a protein of 362 amino acids long (SEQ ID NO.2).

The mouse CRAP homologue was obtained by screening the EST database and aligning the homologous sequences. Human and mouse CRAP are approximately 65% identical and 70% similar on the amino acid level. The mouse sequence is shown in SEQ ID NO. 3.

Exampl 2: s qu nce analysis of the cDNA's

Nucleotide sequence analysis was carried out using dy terminator mix and a 310 Genetic analyzer from Perkin Elmer. The sequence of CRAP is shown in SEQ ID NO.1 whereas the sequence of 4C4 is shown in SEQ ID NO.5.

The CRAP sequence shows a low homology (30% similarity at amino acid level) with Nocturin, a protein that is expressed in the photoreceptor of the eye of *Xenopus laevis* (Green and Beshare, 1996). The partial sequence of the mouse homologue of Nocturin is also known (Puech *et al.*, 1997). Additionally, there is some homology with EST sequences (e.g. genbank EST c23016, aa162513, aa571061, t87026, h45114, aa196281, h94108 and aa337396) and with the C-terminal part of the yeast transcription factor *CCR4* (Malvar *et al.*, 1992). All these homologies are low, and it is clearly unexpected that a human homologue of these proteins would bind to the cytoplasmic domain of CD40.

It is interesting to note that, unexpectedly, CRAP neither 4C4 show any significant homology with TRAF's or other proteins known to interact with CD40.

Example 3: study of the interaction of CRAP protein, 4C4 protein and CRAP protein fragments with other proteins using a yeast two-hybrid interaction assay

The potential binding of CRAP to other proteins was assessed using the yeast two-hybrid assay. The experimental outline is similar to the one described for the two-hybrid screening. Howev r, h re th plasmids for bait,

prey and lacZ report r were transformed simultaneously into the EGY48 yeast strain. Positive interaction was determined either by the growth ph notyp (growth on medium lacking leucine in th presence of galactose, and not in the presence of glucose) or by the blue/white staining on X-gal containing plates (blue colonies only on galactose containing plates, not on glucose containing plates). cDNA's for TRAF2 and for the cytoplasmic regions of CD30, CD40 and TNF-RII were generated by PCR using the pfu polymerase (Promega). PCR fragments encoding RIP, TRADD and FADD were cloned in pCDNA3 (Invitrogen, Carlsbad, CA). cDNA of TRAF3 was obtained from the laboratory of Dixit, Dept Pathol., Univ. Michigan Med. School, MI, USA). The color formation was evaluated as strong and fast (++), strong but slow (+), weak and slow (+/-), none (-) or not determined (nd)

The results for CRAP protein and CRAP fragments are summarized in Table I and Figure 1.

Table I

	CRAP	4F2	4F2d3	4F2d2	4F2d1	4C4	—
CD40	++	++	+/-	+/-	+/-	+	-
CD30	++	++	+/-	+/-	+/-	+	-
TNF-RII	+	+	-	-	-	+	-
LMP-1	-	-	nd	nd	nd	-	-
TRAF2	-	-	nd	nd	nd	nd	-
TRAF3	+	+	-	-	-	nd	-
RIP	++	++	+/-	+/-	+/-	nd	+/-
TRADD	+	nd	nd	nd	nd	nd	-
FADD	-	nd	nd	nd	nd	nd	-
4F2	++	++	-	-	-	+	-
4C4	++	++	-	-	-	+	-

CRAP, as well as th longest CRAP fragment (aa 54 - 362) shows a strong interaction with CD40, CD30, RIP and with 4C4, and a weaker interaction with TNF-RII and TRAF3. Remarkably, CRAP can also self-associate. CRAP fragments, missing the C-terminal end (aa 274 - 362) show only a weak interaction.

4C4 protein is interacting with CD40, CD30, TNF-RII, with the longest fragment of CRAP and with a deletion mutant of TRAF3 which still contains the largest part of the TRAF domain(from aa 380 to the carboxy terminal end of the protein. A smaller form of 4C4 (from amino acid 2 - amino acid 245 in SEQ ID NO.6) is also capable to interact with CD40.

Example 4: expression pattern of CRAP and 4C4

The CRAP gene is widely expressed, as was already indicated by the presence of several partial CRAP cDNA's in the EST sequence data base. The CRAP expression was analyzed by Northern blot analysis against mRNA from different tissues, both from human and mouse (Figure 2). Human CRAP is present as a 2.2 kb transcript in all tissues tested. Besides the 2.2 kb transcript, there is an additional 1.7 kb transcript in a testis sample. (Figure 2A).

Human CRAP expression was further tested and found in the B-cell lines BJAB (Menezes *et al.*, 1975) and DG75 (Ben-Bassat *et al.*, 1977), in the Jurkat T-cell line and in HUVECs.

For mouse CRAP, two transcripts, one of 2.2 kb and one of 3.8 kb can be found on a murine multiple Northern blot (Figure 2B). Mouse CRAP mRNA is also d t cted in all tissues tested, b it to a lower ext nt in skeletal muscl . Both mouse transcripts are not only present in adult animals, but can also be detected in mouse embryo's 7 and 17 days post coitum. These results are an indication that CRAP plays an important role in early development.

On a multiple tissue Northern blot, a 4C4 probe recognizes 3 transcripts, of 1.6kb, 3.5 kb and 7.5kb. All three mRNA's are present in spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes. The expression of the 3.5 kb transcript is most prominent in testis. In ovary, the signal of the 7.5 kb mRNA is strongest.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Novel CD40 interacting proteins

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1920 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:20..1108

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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CTGGATCCTT TATTTCATAA GTTCAGATCA TCTTAAATGA AAATGCCATG ATTATCTGCA
1560

GTTAAGTAGA TGACAGCTAT TCTACATCAG ACTTGATTTT TGTCAGCTAA TTACATAATT
1620

GGTAAGNTAT AATTGAAACC TTATGGCTTA AAATTCCTTA ACTCCTTTTT GATTCATGTT
1680

TGTAGTCATG TTGTCAACAG AGGCAAAGTT AAGCTTGATG ATGGTTAAAA TCGGTTTGAT
1740

AGCACCATGG GACATTTTTT TAACAAAAAT AAATGCATGA AGAGACATAG CCTTTTAGTT
1800

TTGCTAATTG TGAAATGGAA ATGCTTTACA GGAAGTAAAT GCAAATTANT TTTAAGTGTG
1860

CTTTAAAGAA AAATATTTTC CCCACAGGAG AAATTTAAAT AAAGAATTTT ATTTGGTAAA
1920

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 362 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met	Glu	Leu	Gly	Ser	Cys	Leu	Glu	Gly	Gly	Arg	Glu	Ala	Ala	Glu	Glu
1				5				10						15	

Glu	Gly	Glu	Pro	Glu	Val	Lys	Lys	Arg	Arg	Leu	Leu	Cys	Val	Glu	Phe
			20					25					30		

Ala Ser Val Ala Ser Cys Asp Ala Ala Val Ala Gln Cys Phe Leu Ala
35 40 45

Glu Asn Asp Trp Glu M t Glu Arg Ala Leu Asn Ser Tyr Phe Glu Pro
50 55 60

Pro Val Glu Glu Ser Ala Leu Glu Arg Arg Pro Glu Thr Ile Ser Glu
65 70 75 80

Pro Lys Thr Tyr Val Asp Leu Thr Asn Glu Glu Thr Thr Asp Ser Thr
85 90 95

Thr Ser Lys Ile Ser Pro Ser Glu Asp Thr Gln Gln Glu Asn Gly Ser
100 105 110

Met Phe Ser Leu Ile Thr Trp Asn Ile Asp Gly Leu Asp Leu Asn Asn
115 120 125

Leu Ser Glu Arg Ala Arg Gly Val Cys Ser Tyr Leu Ala Leu Tyr Ser
130 135 140

Pro Asp Val Ile Phe Leu Gln Glu Val Ile Pro Pro Tyr Tyr Ser Tyr
145 150 155 160

Leu Lys Lys Arg Ser Ser Asn Tyr Glu Ile Ile Thr Gly His Glu Glu
165 170 175

Gly Tyr Phe Thr Ala Ile Met Leu Lys Lys Ser Arg Val Lys Leu Lys
180 185 190

Ser Gln Glu Ile Ile Pro Phe Pro Ser Thr Lys Met Met Arg Asn Leu
195 200 205

Leu Cys Val His Val Asn Val Ser Gly Asn Glu Leu Cys Leu Met Thr
210 215 220

Ser His Leu Glu Ser Thr Arg Gly His Ala Ala Glu Arg Met Asn Gln
225 230 235 240

Leu Lys Met Val Leu Lys Lys Met Gln Glu Ala Pro Glu Ser Ala Thr
245 250 255

Val Ile Phe Ala Gly Asp Thr Asn Leu Arg Asp Arg Glu Val Thr Arg
260 265 270

Cys Gly Gly Leu Pro Asn Asn Ile Val Asp Val Trp Glu Phe Leu Gly
275 280 285

Lys Pro Lys His Cys Gln Tyr Thr Trp Asp Thr Gln Met Asn Ser Asn
290 295 300

Leu Gly Ile Thr Ala Ala Cys Lys Leu Arg Phe Asp Arg Ile Phe Phe
305 310 315 320

Arg Ala Ala Ala Glu Glu Gly His Ile Ile Pro Arg Ser Leu Asp Leu
325 330 335

Leu Gly Leu Glu Lys L u Asp Cys Gly Arg Phe Pro Ser Asp His Trp

340 345 350

Gly Leu Leu Cys Asn Leu Asp Ile Ile Leu
355 360

(2) INFORMATION FOR SEQ ID NO: 3;

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1312 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:122..1234

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AGCTATTAAT GATTCGAATT TATACGACTC ACTATAGGGA ATTTGGCCCT CGAGGCCAAG
60

AATTCGGCAC GAGGGCGGGA AGCAGCGTGA AGAGCGGGTG TTTTGAGGGG ACCCTGCGGC
120

G ATG GCG TCT GGC AGC AGT TCC GAT GCG GCG GAG CCC GCA GGG CCG
166
Met Ala Ser Gly Ser Ser Ser Asp Ala Ala Glu Pro Ala Gly Pro
1 5 10 15

GCA GGG CGG GCG GCG TCG GCG CCC GAA GCA GCA CAG GCG GAG GAG GAC
214
Ala Gly Arg Ala Ala Ser Ala Pro Glu Ala Ala Gln Ala Glu Glu Asp
20 25 30

CGG GTG AAG AGG CGG CGG CTT CAG TGC CTG GGC TTT GCG TTG GTG GGG
262
Arg Val Lys Arg Arg Arg Leu Gln Cys Leu Gly Phe Ala Leu Val Gly
35 40 45

GGA TGC GAC CCC ACG ATG GTC CCC AGC GTC CTG CGG GAG AAC GAC TGG
310
Gly Cys Asp Pro Thr Met Val Pro Ser Val Leu Arg Glu Asn Asp Trp
50 55 60

CAG ACG CAG AAA GCC CTG AGC GCC TAC TTC GAG CTG CCA GAG AAC GAC
358
Gln Thr Gln Lys Ala Leu Ser Ala Tyr Phe Glu Leu Pro Glu Asn Asp

```
          65                  70                  75

     CAA GGG TGG CCG CGC CAG CCT CCC ACG TCC TTC AAG TCC GAG GCC TAT
     406
 5   Gln Gly Trp Pro Arg Gln Pro Pro Thr Ser Phe Lys Ser Glu Ala Tyr
      80                  85                  90                  95

     GTT GAT CTA ACC AAC GAG GAT GCA AAT GAT ACA ACC ATT TTA GAA GCC
     454
10   Val Asp Leu Thr Asn Glu Asp Ala Asn Asp Thr Thr Ile Leu Glu Ala
                    100                 105                 110

     AGT CCA TCT GGA ACT CCT CTA GAA GAT AGC AGC ACT ATT TCT TTC ATT
     502
15   Ser Pro Ser Gly Thr Pro Leu Glu Asp Ser Ser Thr Ile Ser Phe Ile
                115                 120                 125

     ACC TGG AAT ATT GAT GGA TTA GAT GGA TGC AAT CTG CCC GAG AGG GCT
     550
20   Thr Trp Asn Ile Asp Gly Leu Asp Gly Cys Asn Leu Pro Glu Arg Ala
            130                 135                 140

     CGA GGG GTG TGT TCC TGC CTA GCT TTG TAT AGT CCA GAT GTG GTA TTT
     598
25   Arg Gly Val Cys Ser Cys Leu Ala Leu Tyr Ser Pro Asp Val Val Phe
        145                 150                 155

     CTA CAG GAA GTT ATC CCC CCA TAC TGT GCC TAC CTA AAG AAG AGA GCA
     646
30   Leu Gln Glu Val Ile Pro Pro Tyr Cys Ala Tyr Leu Lys Lys Arg Ala
     160                 165                 170                 175

     GCC AGT TAC ACA ATT ATT ACA GGT AAT GAA GAA GGA TAT TTC ACA GCT
     694
35   Ala Ser Tyr Thr Ile Ile Thr Gly Asn Glu Glu Gly Tyr Phe Thr Ala
                    180                 185                 190

     ATA CTA TTG AAG AAA GGA AGA GTG AAA TTT AAA AGT CAG GAG ATT ATT
     742
40   Ile Leu Leu Lys Lys Gly Arg Val Lys Phe Lys Ser Gln Glu Ile Ile
                195                 200                 205

     CCT TTT CCA AAT ACC AAA ATG ATG AGA AAC CTG CTA TGC GTA AAT GTG
     790
45   Pro Phe Pro Asn Thr Lys Met Met Arg Asn Leu Leu Cys Val Asn Val
            210                 215                 220

     AGT TTG GGT GGA AAT GAA TTT TGC CTT ATG ACA TCC CAT TTG GAG AGC
     838
50   Ser Leu Gly Gly Asn Glu Phe Cys Leu Met Thr Ser His Leu Glu Ser
        225                 230                 235

     ACC AGA GAA CAT TCT GCG GAA CGA ATA AGA CAA TTA AAA ACT GTT CTT
     886
55   Thr Arg Glu His Ser Ala Glu Arg Il  Arg Gln Leu Lys Thr Val Leu
     240                 245                 250                 255
```

```
GGA AAA ATG CAA GAG GCT CCA GAT TCA ACC ACG GTT ATA TTT GCA GGA
934
Gly Lys Met Gln Glu Ala Pro Asp Ser Thr Thr Val Ile Phe Ala Gly
                260                 265                 270

GAT ACA AAT TTA AGA GAT CAA GAA GTT ATC AAA TGT GGT GGT TTA CCT
982
Asp Thr Asn Leu Arg Asp Gln Glu Val Ile Lys Cys Gly Gly Leu Pro
            275                 280                 285

GAC AAC GTT TTT GAT GCC TGG GAA TTT TTA GGC AAA CCT AAA CAT TGC
1030
Asp Asn Val Phe Asp Ala Trp Glu Phe Leu Gly Lys Pro Lys His Cys
        290                 295                 300

CAG TAT ACA TGG GAT ACG AAA GCA AAT AAC AAC CTC AGG ATC CCT GCT
1078
Gln Tyr Thr Trp Asp Thr Lys Ala Asn Asn Asn Leu Arg Ile Pro Ala
    305                 310                 315

GCT TAT AAG CAT CGT TTT GAT CGA ATA TTT TTC AGA GCA GAA GAG GGG
1126
Ala Tyr Lys His Arg Phe Asp Arg Ile Phe Phe Arg Ala Glu Glu Gly
320                 325                 330                 335

CAC CTT ATT CCT CAA AGT TTA GAC CTT GTT GGG TTG GAA AAA CTG GAC
1174
His Leu Ile Pro Gln Ser Leu Asp Leu Val Gly Leu Glu Lys Leu Asp
                340                 345                 350

TGT GGT AGA TTT CCG AGT GAT CAC TGG GGG CTC TTG TGC ACC TTG AAT
1222
Cys Gly Arg Phe Pro Ser Asp His Trp Gly Leu Leu Cys Thr Leu Asn
            355                 360                 365

GTA GTA TTG TGA AAAGCTTCCC ACTTGCAGCT TTACACGTTT GTTAGCACTA
1274
Val Val Leu  *
        370

GTTCTGAATT TGTGTAGGTC TCAACCTTTC AGGACATC
1312
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 371 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
M t Ala Ser Gly Ser Ser Ser Asp Ala Ala Glu Pro Ala Gly Pro Ala
  1               5                  10                  15

Gly Arg Ala Ala Ser Ala Pro Glu Ala Ala Gln Ala Glu Glu Asp Arg
```

```
                20                  25                  30
    Val Lys Arg Arg Arg Leu Gln Cys Leu Gly Phe Ala Leu Val Gly Gly
            35                  40                  45
5
    Cys Asp Pro Thr Met Val Pro Ser Val Leu Arg Glu Asn Asp Trp Gln
        50                  55                  60

    Thr Gln Lys Ala Leu Ser Ala Tyr Phe Glu Leu Pro Glu Asn Asp Gln
10  65                  70                  75                  80

    Gly Trp Pro Arg Gln Pro Pro Thr Ser Phe Lys Ser Glu Ala Tyr Val
                    85                  90                  95

15  Asp Leu Thr Asn Glu Asp Ala Asn Asp Thr Thr Ile Leu Glu Ala Ser
                100                 105                 110

    Pro Ser Gly Thr Pro Leu Glu Asp Ser Ser Thr Ile Ser Phe Ile Thr
            115                 120                 125
20
    Trp Asn Ile Asp Gly Leu Asp Gly Cys Asn Leu Pro Glu Arg Ala Arg
        130                 135                 140

    Gly Val Cys Ser Cys Leu Ala Leu Tyr Ser Pro Asp Val Val Phe Leu
25  145                 150                 155                 160

    Gln Glu Val Ile Pro Pro Tyr Cys Ala Tyr Leu Lys Lys Arg Ala Ala
                    165                 170                 175

30  Ser Tyr Thr Ile Ile Thr Gly Asn Glu Glu Gly Tyr Phe Thr Ala Ile
                180                 185                 190

    Leu Leu Lys Lys Gly Arg Val Lys Phe Lys Ser Gln Glu Ile Ile Pro
            195                 200                 205
35
    Phe Pro Asn Thr Lys Met Met Arg Asn Leu Leu Cys Val Asn Val Ser
        210                 215                 220

    Leu Gly Gly Asn Glu Phe Cys Leu Met Thr Ser His Leu Glu Ser Thr
40  225                 230                 235                 240

    Arg Glu His Ser Ala Glu Arg Ile Arg Gln Leu Lys Thr Val Leu Gly
                    245                 250                 255

45  Lys Met Gln Glu Ala Pro Asp Ser Thr Thr Val Ile Phe Ala Gly Asp
                260                 265                 270

    Thr Asn Leu Arg Asp Gln Glu Val Ile Lys Cys Gly Gly Leu Pro Asp
            275                 280                 285
50
    Asn Val Phe Asp Ala Trp Glu Phe Leu Gly Lys Pro Lys His Cys Gln
        290                 295                 300

    Tyr Thr Trp Asp Thr Lys Ala Asn Asn Asn Leu Arg Ile Pro Ala Ala
55  305                 310                 315                 320

    Tyr Lys His Arg Phe Asp Arg Ile Phe Phe Arg Ala Glu Glu Gly His
                    325                 330                 335
```

L u Ile Pro Gln Ser L u Asp Leu Val Gly Leu Glu Lys Leu Asp Cys
340 345 350

Gly Arg Phe Pro Ser Asp His Trp Gly Leu Leu Cys Thr Leu Asn Val
355 360 365

Val Leu *
370

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1536 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:209..1536

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AGAGAAAGAG GCTCCGGGGA GATAGCGGAC CAGTGAGGGC TGCCCCTCTT TTGAAGCGGT
60

TTTCGTCTCT TTCCGCCAGT GGCCTCCCAG CTCACGCAGG GGCGGGTCCC GGTAGCGCGA
120

GGCGGTGCAG GGCGGGAAGG GGAGTGGTGG CGGCTGCGGC AGTAGGGACA GCAGGAGCAG
180

TGGTGCTGTC AGCGCGGCCG TCGGAGACAT GGGAGACCCG GGGTCGGAAA TAATAGAATC
240

TGTCCCTCCA GCTGGCCCTG AGGCATCTGA GTCAACAACG GATGAAAATG AAGACGACAT
300

TCAGTTTGTC AGTGAAGGAC CATCGAGACC TGTTCTTGAA TACATCGATC TGGTCTGTGG
360

TGATGATGAA AACCCTAGCG CCTATTATAG TGATATTCTG TTTCCTAAAA TGCCAAAACG
420

ACAGGGTGAT TTTTTGCATT TTTTAAATAT GAAGAAGGTG AAAACAGACA CAGAAAATAA
480

TGAAGTGAGC AAAAATCACT GCAGATTGTC TAAGGCAAAG GAACCACATT TCGAGTATAT
540

AGAACAACCA ATCATTGAAG AAAAGCCATC ACTTTCATCA AAGAAAGAAA TAGATAATCT
600

TGTGCTTCCA GATTGTTGGA ATGAAAAACA AGCATTTATG TTTACAGAAC AATACAAATG
660

GCTTGAAATA AAAGAAGGTA AATTAGGATG TAAGGATTGT TCAGCAGTTC GGCATTTGGG
720

ATCGAAAGCA GAAAAGCATG TCCATGTGTC CAAGGAATGG ATTGCATATT TAGTAACCCC
780

TAATGGCAGT AATAAAACTA CTAGGCAAGC TTCTCTACGA AAAAAAATTA GGGAACATGA
840

TGTTTCTAAA GCCCATGGTA AAATTCAGGA TTTGTTAAAG GAATCAACTA ATGATTCAAT
900

TTGTAATTTA GTGCATAAAC AAAATAATAA AAATATTGAT GCTACTGTAA AAGTTTTCAA
960

TACTGTTTAC AGTTTAGTAA AACATAACAG ACCTTTATCT GATATTGAGG GGGCAAGAGA
1020

ATTACAGGAA AAAAATGGAG AGGTAAATTG TTTAAATACA CGTTACAGTG CAACAAGAAT
1080

AGCAGAACAT ATTGCAAAAG AAATGAAGAT GAAGATATTT AAGAATATTA TAGAAGAGAA
1140

TGCCAAAATC TGTATCATAA TTGATGAGGC ATCTACAGTT TCAAAGAAAA CCACCCTAGT
1200

GATTTATCTC CAGTGCACAA TTCAGTCAGC TCCTGCACCT GTTATGTTAT TTGTGGCTTT
1260

AAAAGAATTG GTGTCAACTA TAGCAGAGTG TATTGTCAAT ACATTATTGA CTACTTTAAA
1320

TGATTGTGGT TTTACAAATG AATATTTGAA AGCAAATTTA ATTGCATTTT GTTCTGATGG
1380

TGCTAATACA ANCCTGGGAA GAAAGTCTGG AGTAGCTACA AAATTGTTAG AAAATTTTCC
1440

TGAAATCATC ATTTGGAACT GTTTAAATCA TCGATTACAA TTGTCACTTG ATGATTCTAT
1500

ATCCGAAATA AAACAAATTA ATCATTTAAN NTATAA
1536

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 442 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met	Gly	Asp	Pro	Gly	Ser	Glu	Ile	Ile	Glu	Ser	Val	Pro	Pro	Ala	Gly
1				5				10					15		
Pro	Glu	Ala	Ser	Glu	Ser	Thr	Thr	Asp	Glu	Asn	Glu	Asp	Asp	Ile	Gln
			20					25					30		
Phe	Val	Ser	Glu	Gly	Pro	Ser	Arg	Pro	Val	Leu	Glu	Tyr	Ile	Asp	Leu
		35					40					45			
Val	Cys	Gly	Asp	Asp	Glu	Asn	Pro	Ser	Ala	Tyr	Tyr	Ser	Asp	Ile	Leu
	50					55					60				
Phe	Pro	Lys	Met	Pro	Lys	Arg	Gln	Gly	Asp	Phe	Leu	His	Phe	Leu	Asn
65					70					75					80
Met	Lys	Lys	Val	Lys	Thr	Asp	Thr	Glu	Asn	Asn	Glu	Val	Ser	Lys	Asn
				85					90					95	
His	Cys	Arg	Leu	Ser	Lys	Ala	Lys	Glu	Pro	His	Phe	Glu	Tyr	Ile	Glu
			100					105					110		
Gln	Pro	Ile	Ile	Glu	Glu	Lys	Pro	Ser	Leu	Ser	Ser	Lys	Lys	Glu	Ile
		115					120					125			
Asp	Asn	Leu	Val	Leu	Pro	Asp	Cys	Trp	Asn	Glu	Lys	Gln	Ala	Phe	Met
	130					135					140				
Phe	Thr	Glu	Gln	Tyr	Lys	Trp	Leu	Glu	Ile	Lys	Glu	Gly	Lys	Leu	Gly
145					150					155					160
Cys	Lys	Asp	Cys	Ser	Ala	Val	Arg	His	Leu	Gly	Ser	Lys	Ala	Glu	Lys
				165					170					175	
His	Val	His	Val	Ser	Lys	Glu	Trp	Ile	Ala	Tyr	Leu	Val	Thr	Pro	Asn
			180					185					190		
Gly	Ser	Asn	Lys	Thr	Thr	Arg	Gln	Ala	Ser	Leu	Arg	Lys	Lys	Ile	Arg
		195					200					205			
Glu	His	Asp	Val	S	r	Lys	Ala	His	Gly	Lys	Ile	Gln	Asp	Leu	Leu	Lys
	210						215						220			

Glu Ser Thr Asn Asp Ser Il Cys Asn Leu Val His Lys Gln Asn Asn
225 230 235 240

Lys Asn Ile Asp Ala Thr Val Lys Val Phe Asn Thr Val Tyr Ser Leu
245 250 255

Val Lys His Asn Arg Pro Leu Ser Asp Ile Glu Gly Ala Arg Glu Leu
260 265 270

Gln Glu Lys Asn Gly Glu Val Asn Cys Leu Asn Thr Arg Tyr Ser Ala
275 280 285

Thr Arg Ile Ala Glu His Ile Ala Lys Glu Met Lys Met Lys Ile Phe
290 295 300

Lys Asn Ile Ile Glu Glu Asn Ala Lys Ile Cys Ile Ile Ile Asp Glu
305 310 315 320

Ala Ser Thr Val Ser Lys Lys Thr Thr Leu Val Ile Tyr Leu Gln Cys
325 330 335

Thr Ile Gln Ser Ala Pro Ala Pro Val Met Leu Phe Val Ala Leu Lys
340 345 350

Glu Leu Val Ser Thr Ile Ala Glu Cys Ile Val Asn Thr Leu Leu Thr
355 360 365

Thr Leu Asn Asp Cys Gly Phe Thr Asn Glu Tyr Leu Lys Ala Asn Leu
370 375 380

Ile Ala Phe Cys Ser Asp Gly Ala Asn Thr Xaa Leu Gly Arg Lys Ser
385 390 395 400

Gly Val Ala Thr Lys Leu Leu Glu Asn Phe Pro Glu Ile Ile Ile Trp
405 410 415

Asn Cys Leu Asn His Arg Leu Gln Leu Ser Leu Asp Asp Ser Ile Ser
420 425 430

Glu Ile Lys Gln Ile Asn His Leu Xaa Tyr
435 440

CLAIMS

1. A functional protein, capable of interacting with the cytoplasmic domain of CD40, wherein said protein has no homology to TRAF-proteins.
2. A functional protein according to claim 1, comprising an amino acid sequence with 70-100% homology to the amino acid sequence depicted in SEQ ID NO. 2.
3. A functional protein according to claim 1, comprising an amino acid sequence with 70-100% homology to the amino acid sequence depicted in SEQ ID NO. 4.
4. A functional protein according to claim 1, comprising an amino acid sequence with 70-100% homology to the amino acid sequence depicted in SEQ ID NO. 6.
5. A functional protein according to claim 1, comprising an amino acid sequence with 70-100% homology to the amino acids 274-362 of SEQ ID NO. 2.
6. A functional protein according to claim 1, comprising an amino acid sequence with 70-100% homology to the amino acids 2-245 of SEQ ID NO.6.
7. A nucleic acid encoding a protein according to any of the claims 1-6.
8. A nucleic acid according to claim 7, with about 70-100% homology to the DNA sequence depicted in SEQ ID NO. 1.
9. A nucleic acid according to claim 7, with about 70-100% homology to the DNA sequence depicted in SEQ ID NO.3.

10. A nucleic acid according to claim 7, with about 70-100% homology to th DNA sequ nce depicted in SEQ ID NO. 5.

11. The use of a functional protein, according to any of the claims 1-6 and/or a functional fragment thereof to treat CD40-related diseases and/or NF-κB related diseases.

12. The use according to claim 11 in which the disease is atherosclerosis, arthiritis, multiple sclerosis, systemic lupus erythematosus and/or graft rejection.

13. The use of a functional protein according to any of the claims 1-6 and/or a functional fragment thereof to sensitise tumor cells to anti-tumor treatments.

14. The use of a functional protein according to any of the claims 1-6 and/or a functional fragment thereof to screen for compounds that interfere with the interaction of said protein(s) with other compounds of the CD40 or NF-κB related pathway.

15. A method for screening compounds comprising the use of a protein according to claim 14.

16. A compound isolated with the method according to claim 15.

17. A pharmaceutical composition comprising one or more functional proteins according to any of the claims 1-6 and/or functional fragments thereof and a pharmaceutical acceptable carrier material.

18. A pharmaceutical composition comprising one or more compounds according to claim 16 and a pharmaceutical acceptable carrier material.

19. Use of a protein according to any of the claims 1-6 and/or functional fragments thereof for the manufacture of a pharmaceutical composition to treat CD40 and/or NF-κB related diseases.

ABSTRACT

The present invention relates to novel proteins that interact with the cytoplasmic domain of CD40, which are useful in the treatment of CD40 and/or NF-κB related diseases. Surprisingly, these proteins do not show significant homology with the TRAF-protein family, and offer therefore the possibility to modulate the CD40 and/or NF-kB pathway independently from the TRAF-CD40 interaction.

Figure 1

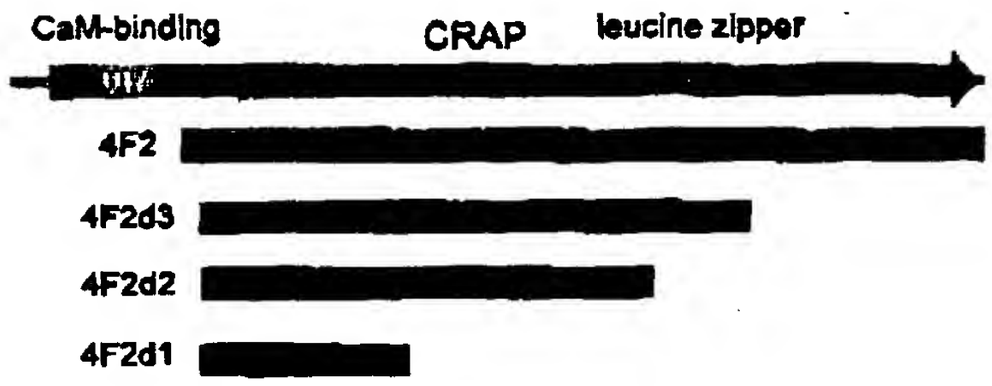
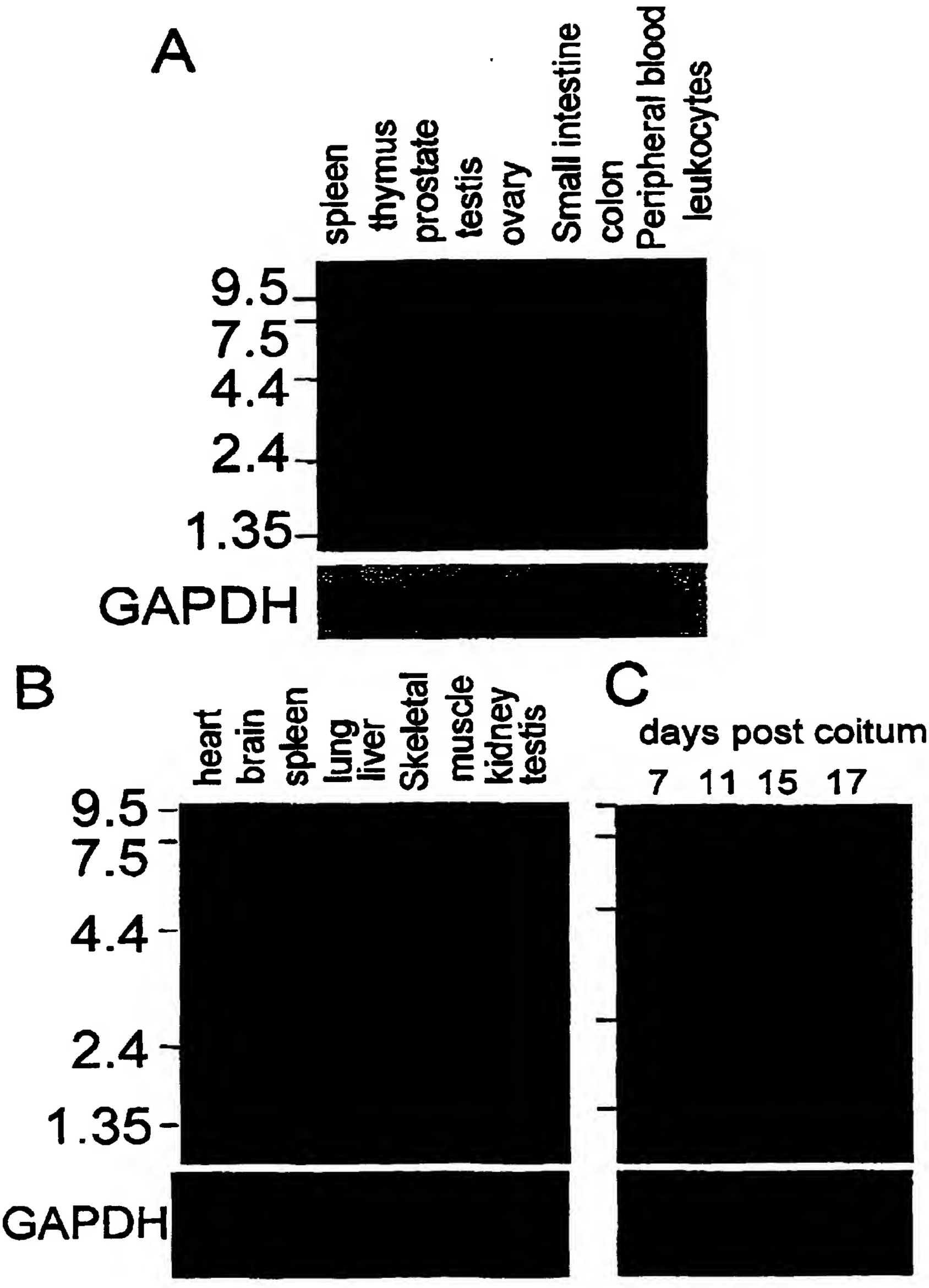


Figure 2


A
spleen
thymus
prostate
testis
ovary
Small intestine
colon
Peripheral blood leukocytes
9.5
7.5
4.4
2.4
1.35
GAPDH
B
heart
brain
spleen
lung
liver
Skeletal muscle
kidney
testis
9.5
7.5
4.4
2.4
1.35
GAPDH
C
days post coitum
7
11
15
17